IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re-application of
Perez et al.

Group art Unit : 1638

Serial N° : 09/380,086

Examiner : Kubelik, S

Filed : November 11, 1999

For : Novel uses of male sterility in plants.

DECLARATION UNDER RULE 132

Hon. Commissioner of Patents and Trademarks
WASHINGTON D.C. 20231

Sir :

I, Pascual PEREZ, residing at 17, Chemin de la Pradelle Varennes, 63450 Chanonat (France) ;

Declare and say :

I am citizen of France.

I am graduated from the University Paul SABATIER (Toulouse, FRANCE) where I got in 1981 a Master degree of Molecular Biology and Biochemistry

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followed by a Graduate Diploma of Applied Studies (DEA) in Microbiology and Microbial Genetics obtained in 1982 in the same Univertsity.

I am currently working as the Laboratory Head Manager and Research Co-ordinator (Corn Transformation & Functionnal Genomic Group leader) in the Biogemma laboratory located , 24, avenue des Landais 63 170 Aubière (France)

I am an inventor of the present patent application and I am aware that the Examiner allegued that using artificial male sterility in plants to prevent transgene dissemination from transgenic plants according to the invention was obvious over Ellstrand et al. and Nyers et al.

However, there was no reasonable expectation of success, and especially no hint of the excellent results that could be obtained.

Trials that support the high efficiency of the invention to prevent transgene escape have been conducted under my personal supervision for corn. I have also got access to the results of our English coworkers belonging our company, Dr Tina Barsby and Dr Wyatt Paul , obtained with modified tobacco plants.

Tobacco carrying a construct confering artificial male sterility (AMS) was field trialed. The constructed included the barnase gene (Hartley, R.W J. Mol. Biol. (1988) 202, 913-915) under the control of the A9 promoter (WO 92 11379). The barnase gene, which encodes a ribonuclease, confers the male sterility, whereas the A9 promoter insures a specific expression of the transgene in the anthers. The cultured modified tobacco proved to be 100% sterile as no pollination could be observed. The invention thereby provides an efficient system for preventing transgene escape.

These experiments further show that male sterility can also be used with great success to avoid transgene dissemination in the context of fruit or seed production. In particular, field trials with corn, modified with different constructs conferring male sterility, allowed seed production as well as

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prevention of pollination of the cultured maize plants. Most of the results coming from different field trials at differents periods and locations are presented in attached Table 1. The transformants described use either the Arabidopsis A9 promoter (WO 92 11379) or the Mac 2 (WO 00 68403) maize promoter for directing Barnase expression in the tapetum and where produced with an *Agrobacterium* mediated transformation method. As presented in the table they have been used at different generation level attesting the stability and efficiency of the male sterile trait and its usefullness to prevent transgene (s) transfert via the male side.

Similar results where obtained with previous transformants obtained with a particle gun transformation procedure using A9 promoter driving the barnase coding region : not presented here.

In the attached table 2 and accompanying figure 1 (see below), it is important to notice that the grain yield of different hybrid versions looks mostly similar with or without the male sterility gene preventing the male diffusion of the basta resistance trait.

As a conclusion, at the time when the invention was reduced to practice, it could not be expected that the use of male sterility would insure such an efficient prevention of transgene escape, nor that male sterility could be applied for the culture of fruit of seed producing plants without prejudice for fruit or seed production.

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Table 1 : RESULTS OF THE OBSERVATION IN FIELD OF TRANSGENIC PLANTS CARRYING THE BARNASE GENE

STB-27b : A9 promoter - Barnase- CaMV terminator and actine promoter - intron actine - Bar- Nos terminator , one event

SMB : Mac2.1 promoter - Barnase - 35 S CaMV terminator and actine promoter - intron actine - Bar- Nos terminator , 6 transformation events

Year	Field trial location	Genetically modified acreage planted (m^2)	Transformation event	Plant Generation	Genotype	Number of kernel sown / trial	% of herbicide tolerant plants	PHENOTYPING OF HERBICIDE TOLERANT PLANTS			
								Number of screened tassels	Number of sterile plants	Number of reduced-fertility plant	Number of fertile plants
1999	Artonne (France)	200	STB-27b (monocopy)	2	87SN165+27b (BC1)	90	53	47	47	0	0
				3	87SN165+27b (BC2)	73	54	32	32	0	0
				4	87SN165+27b (BC3)	624	51	288	285	3	0
	Senasa (Spain)	200		2	87SN165+27b (BC1)	90	52	6	6	0	0
				3	87SN165+27b (BC2)	60	57	4	4	0	0
				4	87SN165+27b (BC3)	600	53	41	41	0	0
2000	Escurolles (France)	200	STB-27b (monocopy)	5	87SN165+27b (BC5)	958	52	100	Sterile (few plants showed abnormal pollen grains)		0
	Monteignet (France)	200		5	87SN165+27b (BC5)	960	51	120	Sterile (few plants showed abnormal pollen grains)		0
	Pau (France)	180		5	87SN165+27b (BC5)	960	49	No pollen shed (Pers. Com. R. Sacaze)			
	Nampa (USA)	20	STB-27b (monocopy)	6	87SN165+27b (BC5)	120	ND	No pollen shed (Final report E. Aubry)			
2000	Lebanon (USA) and Santa Isabel (Puerto Rico)	150	SMB-001a	1	hybride A 188 X 87SN165	30	54	No pollen shed (final report E. Aubry)			
			SMB-005a	1	hybride A 188 X 87SN165	30	42				
			SMB-007a	1	hybride A 188 X 87SN165	30	46				
			SMB-010b	1	hybride A 188 X 87SN165	30	52				
			SMB-011a	1	hybride A 188 X 87SN165	30	52				
			SMB-013b	1	hybride A 188 X 87SN165	30	43				
2001	Artonne (France)	407	STB-27b (monocopy)	8	87SN165+27b (BC7)	1800	51	No pollen shed by the plants (Pers. Com L. Morraud)			

Légend :

Sterile plants : plants that do not present anthesis during male flowering. The spikelets are not well filled and the anthesis do not bear pollen.

Reduced-fertility plants : plants that do not present or do present a few anthesis during male flowering. The anthesis can contain "abnormal" pollen grains or a weak quantity of "looking developped" pollen grain.

Fertile plants : plants that do present a large quantity of anthesis during male flowering with high quantity of fonctional pollen.

Yield evaluation of 5 experimental hybrids produced with the transgenic (male sterile) or non transgenic 87SN165 female line

Yield parameter		Hybrid "Anjou 285"		Hybrid "2447"		Hybrid "Baltimore"		Hybride Anjou 292"		Hybride 250/33"	
		transgenic	conventional	transgenic	conventional	transgenic	conventional	transgenic	conventional	transgenic	conventional
total kernel weight (Kg)	rép. 1	6,50	7,22	8,00	7,20	7,96	7,31	7,60	8,12	8,36	8,00
	rép. 2	8,06	7,82	8,00	8,28	8,00	7,73	8,04	8,09	9,12	9,11
	rép. 3	7,90	7,74	8,76	7,80	7,84	8,60	8,60	9,10	8,38	8,91
	rép. 4	7,62	7,22	9,24	7,46	7,52	6,70	7,50	8,46	8,48	7,02
	Total	**30,08**	**30,00**	**34,00**	**30,74**	**31,32**	**30,34**	**31,74**	**33,77**	**34,34**	**33,04**
	Weight per ear	**0,116**	**0,114**	**0,125**	**0,122**	**0,123**	**0,120**	**0,125**	**0,131**	**0,131**	**0,133**
Weight of 1000 kernels (in grammes, 250 kernels)	rép. 1	71	69	74	71	81	76	66	63	75	71
	rép. 2	75	76	74	74	78	81	67	68	79	79
	rép. 3	71	74	78	73	78	85	71	66	77	79
	rép. 4	74	70	79	74	77	77	68	72	75	78
	Average	**291**	**289**	**305**	**292**	**314**	**319**	**272**	**269**	**306**	**307**
average row number / ear (measure on 12 ears / repeat)	rép. 1	14,33	13,83	13	12,83	12,83	13,83	15,67	15,17	14,67	14
	rép. 2	14	14,17	13,33	13	13,33	13,67	15,67	14,67	14	13,33
	rép. 3	14,17	14,33	13,17	13,17	12,83	13,5	16	15,33	14,33	14,5
	rép. 4	14,33	13,33	12,67	13,67	13,83	13,17	14,67	15	13,83	14,17
	Average	**14,21**	**13,92**	**13,04**	**13,17**	**13,21**	**13,54**	**15,50**	**15,04**	**14,21**	**14,00**
Average kernel / row (analyses on 12 ears, 2 measures)	rép. 1	30,04	28,67	35,92	35,42	32,08	31,00	35,33	34,71	34,54	28,13
	rép. 2	32,33	30,83	33,21	38,17	32,58	29,88	35,63	36,5	33,83	33,75
	rép. 3	28,46	28,5	34,08	36,96	31,17	30,67	35,83	35,87	33,42	33,33
	rép. 4	31,63	31,46	37,79	38,5	32,25	31,58	35,13	33,79	30,71	34,67
	Average	**30,62**	**29,87**	**35,25**	**37,26**	**32,02**	**30,78**	**35,48**	**35,22**	**33,13**	**32,47**

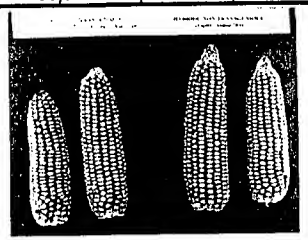
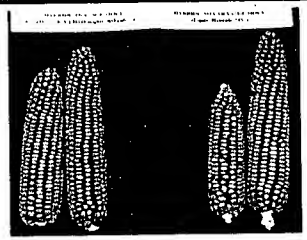
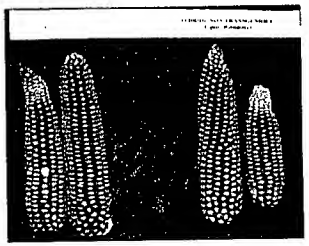
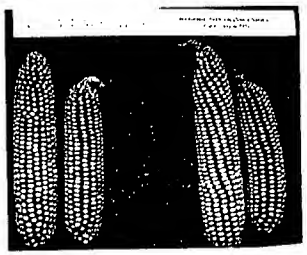
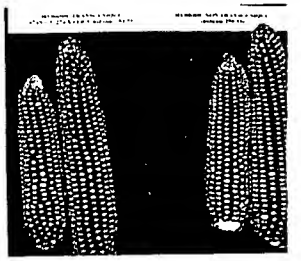
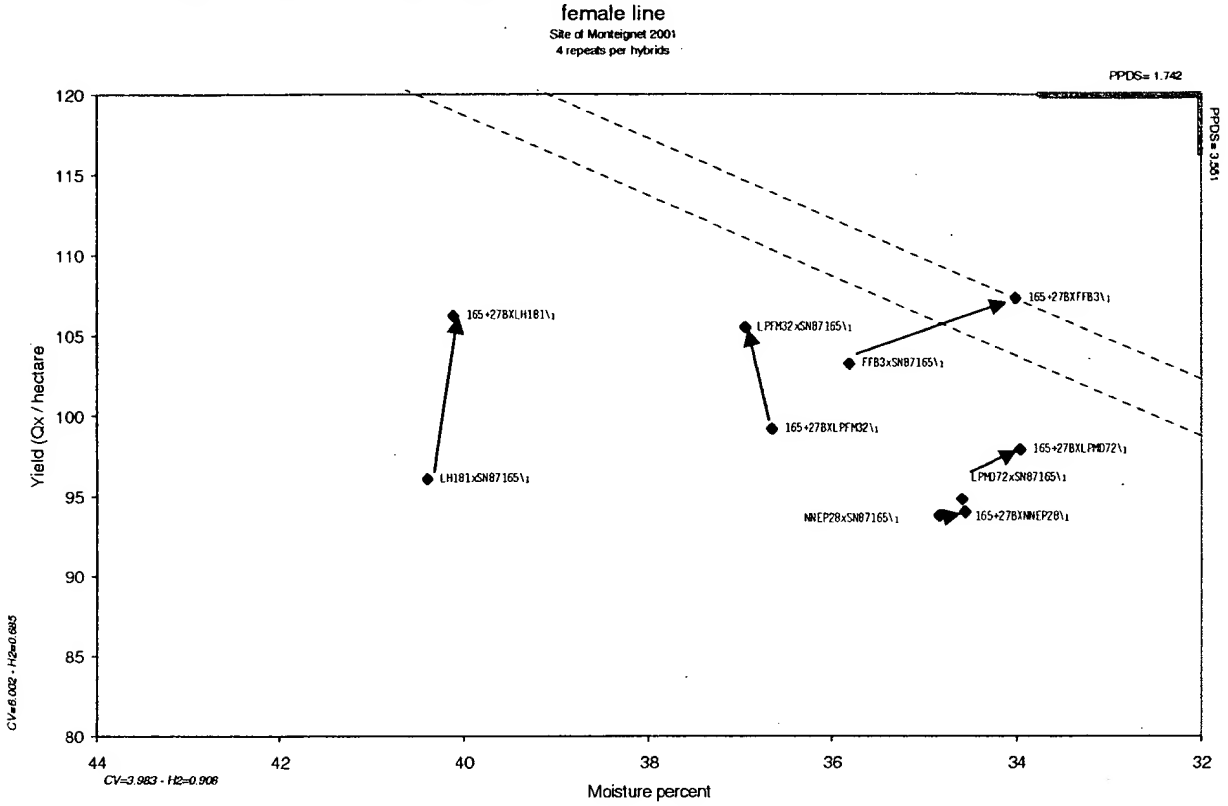






Figure 1 : Comparison between hybrids produced with transgenic and non transgenic 87SN165 female line



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The undersigned Declarant declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true ; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of United States Code and that such wilful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed this 28 th day of February 2002